Bull. Chem. Soc. Ethiop. **2024**, 38(3), 1027-1035.
DOI: https://dx.doi.org/10.4314/bcse.v38i4.17

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

SHORT COMMUNICATION

CHEMICAL CONSTITUENTS OF THE ESSENTIAL OILS FROM *UVARIODENDRON MBAGOI*

Slim R. Juma[1,2], Cosam C. Joseph[1] and Stephen S. Nyandoro[1,*,#]

[1]Chemistry Department, College of Natural and Applied Sciences, University of Dar es Salaam, P.O. Box 35061, Dar es Salaam, Tanzania
[2]Chief Government Chemist Laboratories, P.O. Box 759, Zanzibar, Tanzania

(Received September 8, 2023; Revised February 28, 2024; Accepted March 3, 2024)

ABSTRACT. Several *Uvariodendron* species are known for their scented leaves and stem bark, hence this study. The leaves, twigs, stem and root barks of the newly described *Uvariodendron* species, *U. mbagoi* Dagallier and Couvreur from Kwedijela forest (KF) and Kimboza Forest Reserve (KFR) collected in dry and wet seasons, were steam distilled to yield essential oils that was more abundant in the root bark (2.07-3.85%) than in the leaves, twigs and stem bark (0.09-0.71%). The essential oils from the root bark collected from KF in the wet season (2.07%) was slightly lower than that obtained from the plant part collected from the same locality during the dry season (3.85%). GC-MS analysis indicated that linalool was the most prominent constituent of the leaf (82.18%, KF) and (98.86%, KFR), twig (89.66%, KFR), and stem bark (97.97%, KFR) oil while methyl eugenol (41.08%, KFR) and elemicin (39.28%, KFR) were the major constituents of the root bark oil. The essential oil from the leaves, stem bark, twigs and root bark of *U. mbagoi* exhibited mild to moderate potency when screened for antioxidant, antibacterial, and protease (trypsin) inhibitory activities.

KEY WORDS: *Uvariodendron mbagoi*, Annonaceae, Essential oil, Linalool, Elemicin, Methyl eugenol

INTRODUCTION

Uvariodendron mbagoi Dagallier and Couvreur (Annonaceae) (Figure 1), a plant species endemic to Tanzania, is among the three newly described *Uvariodendron* species occurring in East African coastal forests [1]. The plant is found growing at Kwedijela forest in Handeni District close to the edge of Saadani National Park, at Msata hill in Bagamoyo District and Kimboza Forest Reserve in Morogoro District and known by the vernacular names mchenene, msenene or mkenenne [1]. The two other newly described *Uvariodendron* species, *U. dzomboense* Dagallier, W.R.Q. Luke and Couvreur and *U. schmidtii* W.R.Q. Luke, Dagallier and Couvreur are found in Kenyan coastal forests [1]. The limited distribution of *U. mbagoi* makes it a potentially endangered species, falling under the IUCN criterion B [1]. Its stem barks and twigs, especially when freshly removed from the plant, have a strong bergamot scent [1]. Owing to this pleasant scent, the stem bark is used locally as a spice for flavouring meat meals and for tea [1]. Initially, *U. mbagoi* was wrongly considered to be *U. kirkii* ('mnofu wa kuku' in Kiswahili), which is also endemic to Tanzania and found around the same area as *U. mbagoi* [2]. However, *U. kirkii* does not have any noticeable scent similar to that of *U. mbagoi*.

The genus *Uvariodendron* is confined to tropical forests, mostly in Africa, with five of the species so far identified in Tanzania [1-3]. Several *Uvariodendron* species are known for their scented leaves and stem bark. This has prompted efforts to analyse the plant species for their

*Corresponding author. E-mail: nyandoro@udsm.ac.tz; samnyandoro@yahoo.com
#Dedicated to the memory of the late Professor Mayunga H.H. Nkunya, who passed away when this paper was in the final stages of his great work. His inspiration, dedication and mentorship are greatly admired and will forever be remembered.

essential oil content and to determine the corresponding constituents responsible for the characteristic scents. Thus, analysis of the essential oil from the root bark of *U. calophyllum* R.E.Fr., occurring in Nigeria and Cameroon showed a characteristic predominance of the biosynthetically related sesquiterpenes of bisabolene, santalene and bergamotene categories, while caryophyllene, humulene and their oxygenated derivatives were found abundantly in the essential oil from the wood and stem bark [4]. In another study, the essential oil from *U. connivens* (Benth) R.E. Fr., also occurring in Nigeria and Cameroon, indicated the dominant presence of elemicin, *trans*-cinnamaldehyde and 3',4',5'-trimethoxycinnamyl alcohol [5].

Figure 1. *Uvariodendron mbagoi* stem, twigs and leaves.

The stem and root barks of *U. gorgonis* Verdc., which is one of the *Uvariodendron* species occurring in Tanzania, on chemical investigations yielded eugenol, methyl eugenol and trace amount of dihydroeugenol [6]. Furthermore, GC-MS analysis of the essential oil from the stem bark and leaves of *U. gorgonis* indicated the presence of eugenol as the major constituent, whose abundance (89%) was like the amount occurring in cloves [7]. In another investigation, the stem and root barks of *U. pycnophyllum* (Diels) R.E.Fr., which also occurs in Tanzania, yielded elemicin as the major constituent, together with 3',4',5'-trimethoxycinnamyl alcohol, methyl eugenol, methyl isoeugenol, 2,3-dimethoxy cinnamaldehyde, and a mixture of cinchonain 1a and cinchonain 1b [8]. Among these compounds, methyl eugenol and methyl isoeugenol exhibited activity against *Anopheles gambiae* s.s Giles mosquito larvae, long-term mortality of adult *An. gambiae* mosquitoes when impregnated on bed nets, and mosquito repellence that was more effective than the standard repellent *N*,*N*-diethyl-meta-toluamide (DEET) [9].

Although several other *Uvariodendron* species occurring in Tanzania are known to possess characteristic scents, no bergamot fragrance has been reported from these species, besides that noted for the stem bark and twigs of *U. mbagoi* [1]. Therefore, as part of investigations for the essential oil composition of some Tanzanian indigenous plant species to establish baseline scientific information on the plants' potential as sources of essential oil of economic and biomedical significance, the leaves, twigs, stem bark and root bark of this newly described *Uvariodendron* species, *U. mbagoi*, were analysed for their essential oils composition. The essential oils were screened for their antimicrobial, antioxidant, and protease inhibitory activity. Results from these studies are hereby reported.

EXPERIMENTAL

Plant materials collection. The leaves, twigs, stem barks and root barks of *U. mbagoi* were collected from Kwedijela forest, Kwamsisi village at GPS location 5º51'S, 38º33'E, in Handeni District during the wet (rainy) and dry (none rainy) seasons (in April and October, 2004, respectively) and from Kimboza Forest Reserve at GPS 37 m 368190 utm 9223183, in Morogoro District in the dry season (February, 2005). The plant materials were identified at the Herbarium, Department of Botany at the University of Dar es Salaam where voucher specimens are deposited with reference numbers F. Mbago 3323 and FMM 3333 for April and October 2004 collections from Kwedijela forest, respectively and FFM 3342 for Kimboza collection. Voucher specimen of the latest collection (16th May, 2022, Coll. No. FMM 4207) from Kimboza Forest Reserve is also deposited in the same herbarium. The plant species grows as small tree/shrub up to 4 m tall with flowers born on older stem.

Steam distillation of essential oils. The stem and root barks were dried under shed and then pulverized before steam distillation, while the leaf and twig samples were steam distilled while fresh. The steam-distillation was carried out using Clevenger apparatus. The essential oil obtained upon separation from the water layer was dried over anhydrous sodium sulphate. The percentage yield of the oil was then calculated based on the weight of the respective plant material used.

Determination of the chemical composition of the essential oil samples. The chemical composition of the essential oil samples was determined by GC-MS analysis using a Hewlett-Packard HP 5973 mass spectrometer interfaced with an HP 6890 GC equipment under electron impact ionisation mode at 70 eV and ion source temperature of 240 ºC. The GC equipment was fitted with a 30 m long HP-5 column with internal diameter of 0.25 mm and film thickness of 0.25 µm, and Helium used as the carrier gas with flow rate of 1.21 mL min^{-1}. The oven temperature was programmed to increase from 70 to 325 ºC, being raised steadily at the rate of 4 ºC/min. In cases of unsatisfactory peak separation, the temperature programme was adjusted accordingly. Oil samples (0.2 µL) were injected into the GC-MS equipment. GC chromatograms and mass spectra were recorded, the former was used to determine the percentage composition of the individual constituents using their corresponding peak heights while the mass spectra were used to identify the constituents by matching their mass spectra with those recorded in the Wiley 275 (Wiley, New York) computer library, the associated database, and available literatures.

Antioxidant screening assay. The test reagent was prepared at least 1 h before use by dissolving 2,2-azino-*bis*-(3-ethylbenzo-thioazulene-6-sulfonic acid)-diammonium salt (ABTS, 3.5 mg) in water (1 mL) and stored in a 1.5 mL Eppendorf tube was then mixed with a $K_2S_2O_8$ solution prepared by dissolving the corresponding salt (50 mg) in water (1 mL). The resulting solution (10 µL) was added to the ABTS solution upon which the mixture turned dark green and later after shaking for some minutes becoming nearly black. The latter solution was diluted with water (10 mL) and the resulting solution (200 µL) placed in each of the wells earmarked for the test samples. Ascorbic acid (10 µg/mL) was used as the positive control, which was introduced in the first three wells and marked +, while another set of three wells were left without adding any oil to serve as negative controls. Ascorbic acid (10 µL) used as the positive control decolorized the ABTS solution at once. Each sample (10 µL) was then added in triplicate to the remaining labelled wells filled with ABTS solution (200 µL), making 1:20 v/v concentration of the essential oil. Antioxidant activity was established from the comparison of the colour change as high activity (solution becomes colourless or clear); moderate activity (solution lightens to green colour; and no activity (solution remains dark purple-grey colour).

Antimicrobial screening assay. The agar diffusion method was used with the test medium being prepared by mixing Lysogeny Broth (LB) agar (2.0 g) with water (50 mL) placed in a beaker. The agar solution was heated until the liquid appeared transparent. Agar aliquots (300 µg) were introduced into each well of the 2 x 48-well plate and were left for 5 to 10 min for the gel to solidify. Aliquots (10 µL each) of human saliva placed in a 1.5 mL Eppendorf tube were then pipetted onto the top of the dry agar. Aliquots (10 µL) of a solution of Kanamycin (4 mg) in water (40 µL) previously prepared and stored in a 1.5 mL Eppendorf tube for use as the positive control, were introduced into three impregnated wells marked + (positive control). The subsequent set of three impregnated wells was left without any sample to detect the growth of bacteria and was marked "0" (negative control). The oil samples (10 µL) were introduced into the remaining wells, each in triplicate, and left to stand for about 10 min to allow the agar to absorb the liquids. After drying, the plates were inverted and left at room temperature for 24 h and then the growth of bacteria colonies was recorded.

Protease activity and protease inhibition screening assay. A gelatine-coated strip of radiograph films placed on a flat surface and marked P for protease, W for water, and I for inhibitor were used for the screening test, with the positions for the oil samples being numbered on the strip. A drop of a trypsin solution (10 µL) prepared by dissolving trypsin (7 mg) in water (300 µL) and stored in a 1.5 mL Eppendorf tube, was placed under the P mark on the upper row of the strip, while two drops (10 µL each) of water were placed under the mark W on the strip, one on the upper row and the second on the lower row. This was followed by placing under the I mark two drops of 10 µL each of a previously prepared solution of the inhibitor (3 mg) dissolved in water (30 µL), one on the upper row and the second on the lower row. Two drops (10 µL each) of each test sample were placed under each numerical number, one on the upper row and the second drop on the lower row. An aliquot (10 µL) of the trypsin solution was each placed on the drops of water, inhibitor, and the samples in the lower row of the strip while the upper row was left with the samples only and then allowed to remain undisturbed for 15 min. The strip was then rinsed gently with running water in order to remove any dried-on remnants of the test sample. The upper row of the strip was expected to indicate protease activity by the tested samples showing cleared mark as a result of protein degradation. The lower row indicated protease inhibition (no cleared spot).

RESULTS AND DISCUSSION

The essential oils from the leaves, twigs, stem barks, and root barks of *U. mbagoi* collected from Kwedijela forest and Kimboza Forest Reserve during the wet and dry seasons was obtained by steam distillation. The yield of the oil from the leaves, stem bark and root bark collected from the two localities was almost of the same magnitude for each plant part, the stem bark giving slightly higher yield than the leaves, while the root bark gave the highest yield of oil (Table 1).

The stem bark collected from Kwedijela forest during the wet and dry seasons gave nearly the same yield of essential oil, thus indicating little seasonal influence on essential oil production in the stem bark of *U. mbagoi* occurring in Kwedijela forest. The results also suggested an apparent absence of geographical influence to the essential oil production in the different *U. mbagoi* plant parts from the sampled two localities (Table 1). However, the yield of the essential oil from the root bark collected from Kwedijela forest in the wet season was lower than that obtained from the root bark collected from the same locality during the dry season (Table 1), thus suggesting seasonal influence on the production of essential oil in the root barks. GC-MS analysis on the composition of the essential oils obtained from the leaves, stem and root barks indicated the presence of a variety of compounds at varying abundance levels depending on the plant part and where applicable, geographical location and the season of collecting the materials (either dry or wet season, Table 2). Of the major chemical constituents that occurred more frequently in the

different essential oil samples analysed were linalool (**1**) and methyl eugenol (**2**), which are among the most common constituents of plant-based essential oils [10].

Table 1. Percentage yield of essential oil from different *Uvariodendron mbagoi* plant parts.

Area of collection	Season	Plant part and percentage yield of essential oil			
		Leaves	Twigs	Stem bark	Root bark
Kwedijela forest	Wet	-	-	0.67	2.07
Kwedijela forest	Dry	0.11	-	0.71	3.58
Kimboza Forest Reserve	Dry	0.09	0.53	0.52	3.85

The essential oil obtained from the leaves collected from Kwedijela forest and Kimboza Forest Reserve on GC-MS analysis showed linalool (**1**) as the main constituent (Table 2). Thus, while the essential oil from leaves collected from Kwedijela forest exhibited the presence of linalool (**1**, 82.18%) with linalool oxide (**3**, 9.42%) and *trans*-linalool oxide (**4**, 8.40%) as the only other constituents, the essential oil from the leaves collected from Kimboza Forest Reserve consisted almost exclusively of linalool (**1**, 98.86%), with limonene (**5**), α-humulene (**6**) and β-terpinene (**7**) occurring only in trace amounts (Table 2). Linalool oxide and its isomer *trans*-linalool oxide, which were detected together with linalool in the essential oil from the leaves collected from Kwedijela forest, are also common constituents of other plant-based essential oils [11-13]. These results indicated the leaves, twigs and stem barks of *U. mbagoi* to be important sources of linalool and its oxides, with linalool occurring in the leaves and twigs almost exclusively, and in the stem bark only during the dry season. The results also indicated that the chemical composition of the essential oil from the leaves was not affected much by geographic location of the plant species from which the samples were collected.

Linalool is an unsaturated monoterpene alcohol with a sweet scent similar to that of bergamot oil or French lavender, which occurs as an essential oil constituent in over 200 plant species [11-16]. Although widely used in finished industrial products such as detergents, shampoos, soaps, creams etc., linalool has been shown to exhibit cytotoxicity to human skin cells *in vitro* [11]. Linalool is also the principal component of many essential oils exhibiting a wide variety of biological activities [17-19].

While the main constituent of the essential oil from the stem bark and twigs collected from Kimboza Forest Reserve during the dry season was linalool (97.97% and 89.66%, respectively), the essential oil from the stem bark collected from Kwedijela forest during both the dry and wet seasons showed considerable difference in their linalool content. Thus, while the linalool content of the essential oil from the stem bark collected from Kwedijela forest in the dry season was 62.30% with 2-butylnaphthalene (**8**) being the second major constituent (27.50%), the essential oil from the stem bark collected in the wet season did not have any linalool (Table 2). Instead, the latter essential oil consisted of a complex mixture of compounds, with α-copaene (**9**, 15.99%), *trans*-methylisoeugenol (**10**, 15.25%), caryopyllene oxide (**11**, 10.13%), ß-caryophyllene (**12**, 12.13%), γ-elemene (**13**, 14.86%) and germacrene B (**14**, 14.86%) being the major constituents in the mixture (Table 2).

The wide variation of the chemical constituents of the essential oil obtained from the stem bark collected from Kwedijela forest during the dry and wet seasons could be a result of either seasonal effects or age difference between the plant samples analysed during each of the two seasons. However, this latter factor was not critically explored during these investigations.

The essential oil from the twigs collected from Kimboza Forest Reserve during the dry season also consisted of a large number of other compounds besides linalool, among them β-myrcene (**15**, 1.93%), β-terpinene (**7**, 2.76%) and *trans*-β-ocimene (**16**, 1.63%) being the other main constituents after linalool (Table 2). Since linalool commands a good commercial value in the fragrance industry, the fact that the essential oil from the aerial parts (leaves, twigs and stem bark)

of *U. mbagoi* almost exclusively consists of linalool makes the plant species to be of potential economic value. Furthermore, obtaining it from leaves, highly regenerative parts could be considered praiseworthy for ustainable plant conservation and commercial utilization.

Table 2. Chemical composition of essential oil from the stem bark, twigs and leaves of *Uvariodendron mbagoi* from Kwedijela forest and Kimboza Forest Reserve*.

Compound		% Composition					
	RT	SBKB	SBKD1	SBKD2	TKB	LKD	LKB
Linalool	11:01	97.97	62.30	0.00	89.66	82.18	90.86
Limonene	9:08	0.00	2.69	0.00	0.00	0.00	0.71
α-Copaene	17:29	0.00	1.85	15.99	0.00	0.00	0.00
Methyl eugenol	18:22	0.00	0.00	6.27	0.00	0.00	0.00
α-Humulene	19:05	0.00	0.00	2.62	0.00	0.00	0.25
α-Pinene	7:06	0.00	0.00	0.00	0.29	0.00	0.00
α-Phellandrene	8:52	0.00	0.00	0.00	0.35	0.00	0.00
α-Terpinene	9:08	0.00	0.00	0.00	0.29	0.00	0.00
α-Terpineol	13:23	0.00	0.00	0.00	0.18	0.00	0.00
o-Cymene	9:00	0.11	0.00	0.00	0.53	0.00	0.00
trans-Methyl isoeugenol	18.14	0.00	0.00	17.25	0.00	0.00	0.00
δ-Cadinene	20:25	0.13	2.73	2.14	0.00	0.00	0.00
Caryophyllene oxide	22.12	0.00	0.00	10.13	0.00	0.00	0.00
β-Myrcene	8:05	0.13	0.00	0.00	1.93	0.00	0.00
β-Terpinene	9:37	0.10	0.00	0.00	2.76	0.00	0.18
trans-β-Ocimene	9:42	0.00	0.00	0.00	1.63	0.00	0.00
β-Elemene	17:55	0.00	0.00	1.88	0.00	0.00	0.00
β-Caryophyllene	18:29	0.00	0.00	12.13	0.00	0.00	0.00
Linalool oxide	10:56	0.12	0.00	0.00	0.00	9.42	0.00
Linalyl acetate	11:01	0.00	0.00	0.00	0.21	0.00	0.00
trans-Linalool	11:31	0.00	0.00	0.00	0.00	8.40	0.00
2-Butylnaphthalene	8:34	0.00	27.50	0.00	0.00	0.00	0.00
α-Ylangene	17:16	0.00	2.94	0.00	0.00	0.00	0.00
γ-Elemene	17:50	0.00	0.00	14.86	0.00	0.00	0.00
γ-Terpinene	10:16	0.00	0.00	0.00	0.43	0.00	0.00
Germacrene B	20:28	0.00	0.00	14.86	0.00	0.00	0.00
β-Eudesmol	33:20	0.00	0.00	4.36	0.00	0.00	0.00
α-Eudesmol	32:59	0.00	0.00	3.28	0.00	0.00	0.00

* SBKB = Stem bark Kimboza Forest Reserve dry season; SBKD1 = Stem bark Kwedijela forest dry season; SBKD2 = Stem bark Kwedijela forest wet season; TKB = Twigs Kimboza Forest Reserve dry season; LKD = Leaves Kwedijela forest dry season; LKB = Leaves Kimboza Forest Reserve dry season, RT = Retention time (mins)

The essential oil obtained from the root barks collected from Kimboza Forest Reserve during the dry season on GC-MS analysis indicated the presence of a large number of compounds compared to the essential oil from the root barks collected from Kwedijela forest during the same season. The major constituents of the essential oil from the root bark collected from both localities were methyl eugenol (**2**) and elemicin (**17**), which occurred together with their corresponding *trans*-isomers **10** and **18**, respectively (Table 3). The presence of a large amount of elemicin (**17**) in the essential oil from the root bark of *U. mbagoi* collected from Kwedijela forest during the dry season suggests the possibility of biochemical transformation of *trans*-methyl isoeugenol (**10**) into elemicin (**17**) during the dry season. This is because the amount of *trans*-methyl isoeugenol (**10**) decreased substantially from 30.09% during the wet season to only 3.98% in the dry season, while

the amount of elemicin was higher during the dry season (29.96%) than in the wet season (11.42%, Table 3). Elemicin (**17**), which is a constituent of several essential oils including oil of nutmeg, is believed to be responsible for the subtle psychoactive effects of nutmeg [20]. Both methyl eugenol and *trans*-methylisoeugenol are common constituents of plant-based essential oils and are widely used as fragrance ingredients in perfumes, toiletries and detergents [11]. Structures of the major compounds in the essencial oils of *U. mbagoi* are shown in Figure 2.

Table 3. Chemical constituents of essential oil from the root bark of *Uvariodendron mbagoi* from Kwedijela forest and Kimboza Forest Reserve*.

Compound		% Composition			Compound		% Composition		
	RT	RKD1	RKD2	RKB		RT	RKD1	RKD2	RKB
Camphene	7:26	1.55	1.31	2.61	Elemicin	21:26	29.96	11.42	39.28
Limonene	9:08	0.63	1.05	0.00	Caryophyllene oxide	22:12	0.00	0.55	0.48
Linalool	11:01	0.59	2.98	1.10	Viridiflorol	8:45	5.40	6.51	0.00
α-Copaene	17:29	0.79	0.26	1.52	Valencene	23:19	2.85	3.25	1.73
Methyl eugenol	18:22	28.52	33.15	41.08	*trans*-Isoelemicin	21:20	19.65	2.57	0.00
α-Humulene	19:05	0.00	0.72	0.50	β-Terpinene	9:37	0.00	0.00	1.29
trans-Methyl isoeugenol	18:14	3.98	30.09	0.00	β-Elemene	17:55	0.00	0.00	0.46
δ-Cadinene	20:26	0.56	0.54	1.30	β-Caryophyllene	18:29	0.00	0.00	2.03

* RKD1 = Kwedijela forest dry season; RKD2 = Kwedijela forest wet season; RKB = Kimboza Forest Reserve dry season.

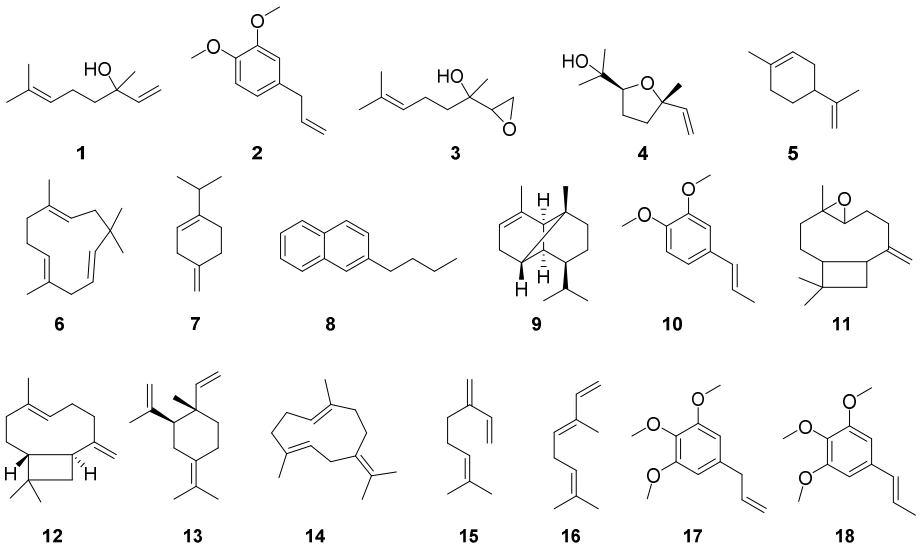

Figure 2. Structure of the major compounds in the essencial oils of *U. mbagoi*.

In the biological screening tests (Table 4), the essential oils from the leaves, stem barks, twigs and root bark of *U. mbagoi* exhibited moderate antioxidant activity at 1:20 v/v concentration of the essential oil/ABTS changing ABTS solution to light green colour. The essential oils from leaves, stem barks and twigs exhibited mild antimicrobial activity. The oils from the stem barks

and twigs also exhibiting mild protease inhibitory activity at the corresponding screening concentrations. In the literature, the phenylpropenoids such as *O*-methyleugenol, *O*-methylisoeugenol and 2,3-dimethoxy-cinnamaldehyde were isolated as the mosquitocidal constituents of the stem and root bark extracts of *U. pycnophyllum* [9]. As stated earlier, linalool that was identified as the principal component of the investigated plant essential oils is known for its wide variety of biological activities [17-19]. Therefore, these previous findings and our results further indicate *Uvariodendron* species to be potential sources of bioactive essential oil constituents alongside other potential applications including in cosmetic industries.

Table 4. Biological activities screening.

Sample	Antioxidant activity	Antimicrobial activity	Protease activity	Protease inhibition activity
LKB	++	+	-	-
SBKB	++	+	-	+
RKB	++	-	-	-
TKB	++	+	-	+
LKD	++	+	-	-
SBKD1	++	+	-	+
RKD1	++	-	-	+
RKD2	++	+	-	+

Key: - Not active, + mildly active (inhibitory effect less than 5 mm), ++ moderately active (ABTS solution lightens to green colour).

CONCLUSION

Hydro-distilled essential oils from the leaves, twigs, stem and root barks of *Uvariodendron mbagoi* from Kwedijela forest and Kimboza Forest Reserve, collected in dry and wet seasons yielded essential oils with higher amount in the root bark than other parts of the plant, albeit with insignificant seasonal and geographical localities influences. Linalool was established to be the major constituent of the leaf, twig, and stem bark essential oils while methyl eugenol and elemicin were the main components of the root bark oils. The essential oils from the investigated plant exhibited mild to moderate antioxidant, antibacterial, and protease in inhibitory properties. This work therefore further indicates *Uvariodendron* species to be potential sources of essential oils of biomedical and economic significance.

ACKNOWLEDGEMENTS

The authors express sincere appreciations to the Zanzibar State Trading Corporation (ZSTC) for financial support extended on behalf of the Government of Zanzibar in facilitating these investigations, the Government Chemist Laboratory Agency of the United Republic of Tanzania for availing the GC-MS equipment used in these studies and the corresponding technical support. Our gratitude to the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences for the bioassays, and the Department of Chemistry, University of Dar es Salaam for availing the required research materials and facilities. Mr. Frank M. Mbago, the Herbarium Curator, Botany Department, University of Dar es Salaam is sincerely acknowledged for locating and identifying the plant species, and for collecting the investigated plant materials.

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